

## Detection of Bocavirus in Saliva of Children with and without Respiratory Illness<sup>∇</sup>

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**We evaluated saliva samples from 149 children 2 to 11 years old for human bocavirus (hBoV) DNA. hBoV was detected in saliva samples at asymptomatic enrollment in 3% (5/149) and during respiratory illness in 2% (2/106) of the cases. hBoV was detected in only 1/149 asymptomatic and 0/106 illness nasal samples.**

Human bocavirus (hBoV) is commonly detected in young children with respiratory symptoms but also in children with symptoms including vomiting and diarrhea. hBoV detection in stool and blood (2, 12) indicates that hBoV may infect sites outside the respiratory tract. We hypothesized that hBoV would be detected in saliva samples.

Samples were obtained from 149 children 2 to 11 years old in King County, WA, who were enrolled between November 2007 and March 2008 for a study of respiratory illness. Nasal and saliva samples were collected at enrollment, when the children were asymptomatic, and again at the onset of respiratory illness, up to 120 days after enrollment. Respiratory illness was defined as parental report of “developing a cold” and at least one of the following respiratory symptoms: runny nose, nasal congestion, sneezing, or coughing. Nasal specimens were collected by deep nasal swab using flocced-tip Copan swabs, which were immediately submerged in a vial containing 0.5 ml lysis buffer and stored at room temperature (5). Nucleic acid extraction of nasal samples was performed as previously described (9). Sixty microliters of saliva was collected by placing five strips of sterile Schirmer test filter paper into the child's mouth as previously described (14). Saliva samples were transported on ice and stored at  $-20^{\circ}\text{C}$  or colder. The sample ends of five filter paper strips, each containing 12  $\mu\text{l}$  of saliva, were digested overnight in AVL tissue lysis buffer and proteinase K (Qiagen Corp) as previously described (14). Nucleic acid extraction of saliva samples was performed with Qiagen spin columns and reagents according to the manufacturer's instructions, with samples eluted in 200  $\mu\text{l}$  water.

Extracted DNA was tested first qualitatively and again quantitatively for hBoV by using a real-time PCR assay targeting the NP1 gene (3). Quantitative results were obtained by comparing specimen PCR threshold cycle values to a standard curve generated by amplifying known copy numbers of a plasmid containing the hBoV amplicon. One thousand copies/reaction mixture of EXO DNA, which is derived from jellyfish,

was added to both specimen extractions, and EXO primers and probe were added to the PCR mixtures to monitor for false-negative PCR results due to inefficient extraction or amplification inhibitors (10). Nasal swab samples were also tested for known respiratory viruses, including respiratory syncytial virus, human metapneumovirus (hMPV), influenza, parainfluenza, adenovirus, rhinovirus (RhV), and coronavirus, by using previously published assays (6–9, 11). The frequency of hBoV detection at enrollment was compared with hBoV detection at the time of illness by using McNemar's test.

All asymptomatic enrollment samples ( $n = 56$ ) and respiratory illness samples ( $n = 49$ ) from the 2- to 4-year-old children were tested. Only respiratory illness saliva samples from the 5- to 11-year-old children were tested ( $n = 57$ ). Overall, we detected hBoV in seven saliva samples collected from six children (Table 1). hBoV was detected in the saliva of 5 (9%) of 56 enrollment samples from asymptomatic children 2 to 4 years old. Two of the five children with a positive saliva test at enrollment did not have a respiratory illness in the 120-day follow-up period. hBoV was detected in saliva at the onset of two illnesses, those of an afebrile 2-year-old child with moderate upper respiratory symptoms beginning 18 days after enrollment and a 7-year-old child with fever and a severe runny nose and cough beginning 13 days after enrollment. The nasal samples corresponding to detection of hBoV in both saliva specimens were hBoV negative. hBoV detection in saliva was not associated with illness by McNemar's test ( $P = 0.56$ ;  $n = 49$  2- to 4-year-old children).

Only one nasal sample was hBoV<sup>+</sup>. This was collected at enrollment from an asymptomatic 3-year-old child who did not have a subsequent respiratory illness during the follow-up period. The corresponding saliva sample was also hBoV<sup>+</sup>. This low prevalence in nasal samples could be due to the relatively greater age of our subjects and the relatively short follow-up time. While hBoV has been detected in older children, seroprevalence data indicate that most children have experienced their primary hBoV infection by 4 years of age (4). Our low detection rate is unlikely to be caused by nasal sample collection or inhibition, as hMPV and RhV were detected in hBoV<sup>-</sup> nasal samples from children with hBoV<sup>+</sup> saliva samples.

Viral loads ranged from 2,070 to 85,800 copies of hBoV per

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TABLE 1. Bocavirus detection in asymptomatic children and children with respiratory illness

Subject no.	Age (yr)	Asymptomatic enrollment				Respiratory illness			
		Saliva	Copies/ml saliva	Nasal	Copies/swab	Days to Illness	Saliva	Copies/ml saliva	Nasal
1	2	+ <sup>c</sup>	10,200	— <sup>d</sup>		19	+	3,010	— (RhV <sup>+</sup> )
2	2	+	ND <sup>a</sup>	—		NI <sup>b</sup>	NI	NI	NI
3	4	+	2,070	— (RhV <sup>+</sup> )		4	—		— (RhV <sup>+</sup> )
4	3	+	13,500	—		32	—		— (hMPV <sup>+</sup> )
5	3	+	85,800	+	107,000	NI	NI	NI	NI
6	7	—		—		13	+	9,010	— (hMPV <sup>+</sup> )

<sup>a</sup> ND, none detected.

<sup>b</sup> NI, no illness after 120 days.

<sup>c</sup> +, positive.

<sup>d</sup> —, negative.

ml of saliva. One saliva sample that was positive by the qualitative assay was negative by quantitative assay but was detected by real-time PCR at a cycle threshold value of 38.3, indicating a low viral load. The mean log saliva viral load appeared higher when no respiratory virus was detected in the nasal sample (4.36 log copies of hBoV/ml in three samples versus 3.59 log copies/ml in three samples), although the total sample numbers were small. A lower hBoV viral load in the presence of other viruses has been previously reported (1) and could possibly represent extended asymptomatic hBoV shedding from a previous illness that had clinically resolved.

Our detection of hBoV in the saliva of children without respiratory illness and without hBoV in corresponding respiratory samples illustrates that detection of hBoV in a single sample does not necessarily indicate an active respiratory illness. hBoV detection in asymptomatic children may be due to prolonged shedding from a previous illness, especially in view of reports of viral shedding for up to 4 months after respiratory illness (13). hBoV present at a high viral load in respiratory epithelium could potentially cause detectable levels of virions to be shed into the oral cavity. However, we would then expect nasal samples obtained at the time of positive saliva sample collection to also be positive, which we did not observe in five of six cases.

Our findings do not support a causal relationship between hBoV and respiratory disease and show that the virus can be detected outside of the respiratory tract, even in asymptomatic children. Our novel saliva testing method for hBoV is convenient and noninvasive and may be useful in future studies of this new virus.

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